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Heat tolerance of dairy lactococcal c2 phages

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Introduction and aim

Lactococcus lactis strains are the most important starters in the dairy industry and bacteriophage attack is the major cause of fermentation failures. In spite of this, there is little detailed knowledge on the heat tolerance of different phages.

The aim of the present study was to characterize the thermal tolerance and inactivation kinetics of nine lactococcal c2 phages.

Materials and methods

Phages: dairy isolates, classified as c2 by electron microscopy and PCR, different host range.

Enumeration: double-layer plaque assay method.

Suspension medium for thermal inactivations: skimmed milk.

Screening for thermal resistance: 1.5 mL screw-capped stainless steel tubes submerged in a water-bath. Heat treatments at 65°C-80°C, holding time 5 min.

Inactivation kinetics: two phages (P635 and CHPC670) using the coil method (Sherwood Instruments).

Transmission electron micrographs were performed on 0, 15 and 25% thermally inactivated phages.

Results

In the screening pronounced variation was found.

Three phages (P220, CHPC227 and CHPC670) displayed high sensitivity resulting in >8 log reductions after 70°C for 5 min, while the most thermally resistant phages (P635, P109, CHPC134, CHPC180 and P132) required 80°C for 5 min to obtain the same reduction. One phage with intermediate heat resistance was observed (P684).

No correlation between thermal resistance and time period of phage isolation was found.

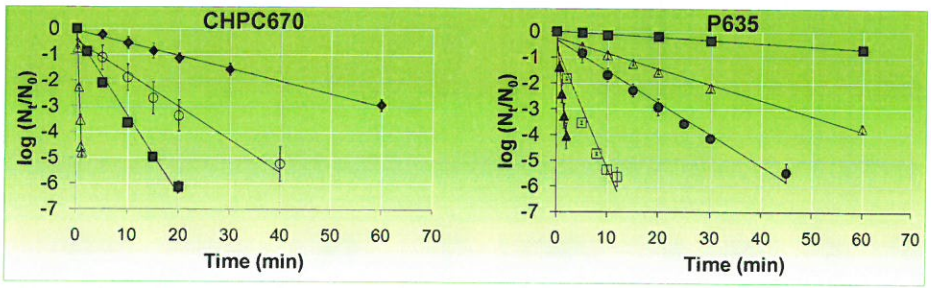

Fig. 2. Inactivation kinetics of a heat sensitive (CHPC670) and heat resistant c2 phage (P635) after treatment at 60°C (◆), 63°C (○), 65°C (■), 70°C (Δ), 72°C (●), 75°C (□), and 78°C (▲) in skimmed milk. Error bars represent the standard derivation of triplicate determinations.

Table 1: D- and z-values for phages P635 and CHPC670.

Phage	Temperature	D-value (min)[a]	z-value (°C)[a]
P635	65°C	86.4 ±14.0	6.0 ±0.1
	70°C	16.6 ±0.7	
	72°C	8.5 ±0.6	
	75°C	2.8 ±0.8	
	78°C	0.5 ±0.0	
CHPC670	60°C	20.5 ±1.2	5.1 ±0.1
	63°C	7.9 ±0.3	
	65°C	3.9 ±0.2	
	70°C	0.2 ±0.0	

[a]Values are given with standard error.

Inactivation kinetics followed first order.

D_{70}- values of 12 sec and 16.6 min were calculated for the sensitive and the resistant phage, respectively.

Loss of phage DNA from capsids and disintegration of phages into head and tail structures were observed after 15% inactivation. In addition, aggregation of phage tails was observed after 25% inactivation.

Observed percentage of phages with morphological changes correlated with the degree of phage inactivation as measured by pour plate titration.

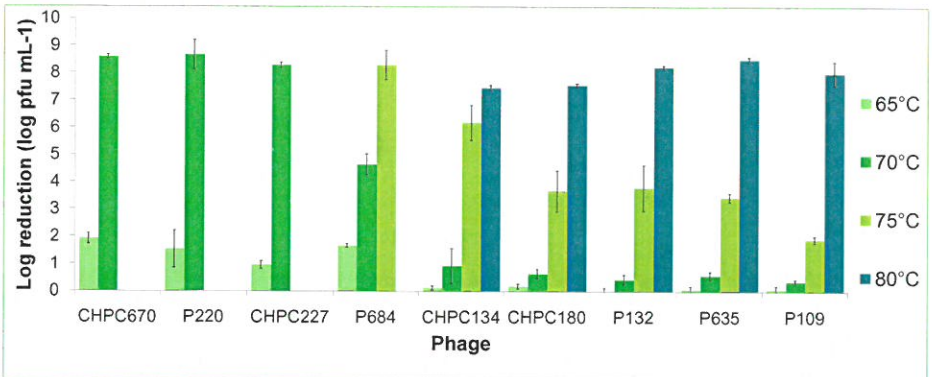

Fig. 1. Inactivation of nine *Lactococcus lactis* c2 phages after treatment at 65°C, 70°C, 75°C, and 80°C for 5 min in skimmed milk. When total inactivation was obtained (8-log reductions), higher temperatures were not tested. Error bars represent the standard derivation of triplicate determinations.

Conclusion

- **Complete inactivation of lactococcal c2 phages was observed within a temperature span of at least 10°C.**
- **Many c2 phages will survive pasteurization temperatures (72°C, 15 sec).**
- **Thermal inactivation of c2 phages follows first-order kinetics with instant heating.**
- **First step in thermal inactivation of P635 is DNA release and disintegration of head and tails.**

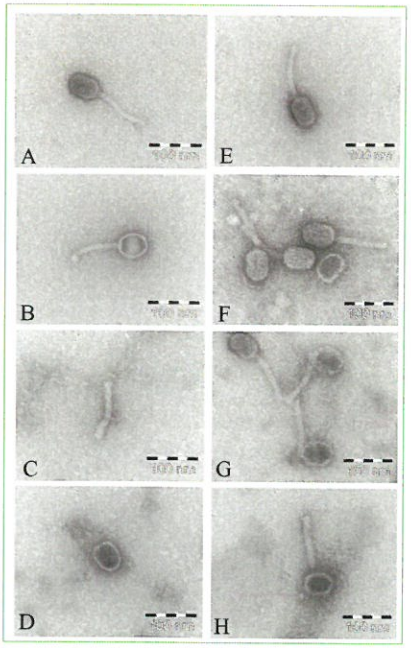

Fig. 3. Transmission electron micrographs of phage P635 before (A+E) and after either 15% inactivation (B-D) or 25% inactivation (F-H) by heat treatment at 65°C. Scale bar indicates 100 nm.

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